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Assessment of Microbiological Quality and the Anti-Bacterial Traits of Sterile Liquids Used for Medication of Eye and Ear Infections in Bangladesh

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ABSTRACT

Current study endeavored to examine the microbiological quality as well as the anti-bacterial traits of different sterile liquid drops commonly applied for the treatment of eye and ear infections in Bangladesh. Fifteen (15) different types of liquid drops manufactured in different pharmaceutical industries were microbiologically examined through common, traditional and replicable cultural and biochemical tests. Total viable bacterial and fungal load (~10^1 -10^4 cfu/ml) showed repugnant contamination of which 7 samples significantly exceeded United States Pharmacopeia (USP) or British Pharmacopeia (BP) limit (<10^2 cfu/ml). While the fecal coliforms and *Escherichia coli* were absent in all samples, the prevalence of *Staphylococcus* spp. and *Bacillus* spp. was scored in 80% and 60%, respectively in all samples. Two samples were found to harbor *Klebsiella* spp., and *Pseudomonas* spp. Samples were also analyzed for antimicrobial activity against different pathogenic bacteria on Muller-Hinton agar. Thirteen (13) samples (88.66%) exhibited the anti-bacterial activity up to a zone of inhibition size of 42.5 mm against almost all pathogens tested.

Key Words: Eye & Ear drops, microbiological quality, antimicrobial activity, public health.

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INTRODUCTION

Drug-borne human infections upon usage of contaminated pharmaceutical preparations are not unlikely around the globe[1-3]. Among the pharmaceutical products, eye & ear drops are mostly sterile aqueous or oily solutions/ suspensions consisting of one or more active ingredients with the addition of preservatives intended to exclude microbial contamination[4-7]. Microbial contamination including *Clostridium tetani* and *Pseudomonas aeruginosa* infections, fungal or even viral infections from raw materials as well as the water used for the manufacturing of pharmaceutical drugs are suggestive of the unexplained treatment complications in patients [2,8-13]. Presence of contaminating microorganisms in eye drops and other ophthalmic solutions with an exceeding limit of $<10^2$cfu/ml in eye and ear drops, raises the public health risk[14-24].

Eye diseases are common in patients residing in long-term care facilities[12,19,25,26]. Besides, bacterial contagion of eye drops may alter the pH of the solution resulting in reduction of drug efficacy[27]. However, use of preservative has been reported to mitigate such problems[21,28-32].

Appropriately equipped and microbiologically controlled manufacturing and packaging facilities principally account for effective drug management[33]. Nevertheless, microbiological monitoring of sterile pharmaceutical drugs during distribution and storage are inadequate in Bangladesh[34-35]. Along with the ongoing research on other infective diseases, proper investigation in eye and ear related disease diagnosis is also demanding to ensure the overall public health remedies[36-37]. Moreover, the problems of the increasing commencement of drug-resistance as well as the reduction of drug potency may urge the necessity of screening of the associated antimicrobial activity[38]. Based on these facts, present study (1) assessed the contamination extent within the randomly collected eye and ear drops in their finished stages, and (2) also demonstrated their antimicrobial activity.

MATERIALS AND METHODS

Study area, sample collection & processing, and microbiological examination

Fifteen (15) different eye and ear drop samples were collected from different retailer drug stores in Dhaka city during September 2013 – November 2013, and were subjected to microbiological examination. Isolation and enumeration of total viable bacteria and fungi was conducted. Presence of specific pathogens was monitored as well[39,40].

Enumeration of total viable bacterial and fungal count

Ten (10) ml of samples were homogeneously mixed with 90 ml of buffer peptone water (BPW), and serial dilutions were prepared up to 10^{-}. An aliquot of 0.1 ml of each suspension from the 10^{-}

^{2} was spread onto Nutrient agar (NA) plate to enumerate the total bacteria (TVB) and on Sabouraud dextrose agar (SDA) plate for the estimation of fungal load. Then the NA and SDA plates were incubated at 37 ^{o}C for 24 hours and at 25 ^{o}C for 48 to 72 hours, respectively.

Enumeration of specific pathogens

0.1 ml from the 10^{-2} dilution of each sample was spread onto Membrane fecal coliform (MFC), Mac Conkey agar, Mannitol salt agar (MSA), Pseudomonas agar, S-S (Salmonella-Shigella) agar and Mannitol egg yolk polymyxin (MYP) agar base media for the enumeration of total fecal coliform, *Escherichia coli*, *Staphylococcus* spp, *Pseudomonas* spp, *Salmonella* spp, *Shigella* spp and *Bacillus* spp., consecutively. All the plates were incubated at 37 ^{o}C for 24 hours except MFC agar which was incubated at 45 ^{o}Cfor 18-24 hours. Confirmative identification of the specific pathogens was accomplished through the biochemical tests[40]. Presence of *E.coli*, *Bacillus* spp, and *Staphylococcus* spp was further confirmed by the Gram staining procedure.

Assay of antimicrobial activity

All the samples were evaluated for antimicrobial activity against *E. coli*, and *Pseudomonas aereginosa* in Muller Hinton agar plates[39]. Additionally, the laboratory isolates of *Bacillus* spp, *Salmonella* spp, *Vibrio* spp, *Listeria* spp, *Staphylococcus* spp and *Klebsiella* spp. were also used as the test microorganisms. Using sterile technique, a small portion (100 µl) of the suspension of the test microorganisms was added on the plates with a sterile cotton swab and bacterial lawns were prepared. Then the wells (8 mm) were prepared using a cork borer, to which 0.1 ml (11µg/µL) of sample was poured[39]. Gentamicin 10 µg & Streptomycin 10 µg were used for samples (1-6) & (7-15) correspondingly as the positive control.

RESULTS AND DISCUSSION

Microbial safety of a particular pharmaceutical product application solely depends on its manufacturing and packaging conditions followed by further distribution, storage and aseptic usage. Current study figured out such microbial safety within the eye and ear drops which could be significant health concern. The study showed that all the products analyzed harbored viable bacteria above the recommended microbial limit which broadens the challenge for stringent jurisdiction over the processing of such products for the sake of public health management.

Prevalence of microorganisms in the eye and ear drops

From the current study, the majority of the tested samples were found to be highly contaminated with bacteria and fungi. The presence of total viable bacteria in 7 (46.7%) out of 15 eye and ear drop samples exceeded the USP or BP limit (<10^{2}cfu/ml); rest of the samples also showed the

bacterial prevalence; however within the USP or BP limit (Table 1). The prevalence of fungi in the samples was within the limit specified by USP or BP. Examination for the presence of Gram negative pathogens showed the complete absence of fecal coliforms, *E. coli*, *Salmonella* spp, *Shigella* spp. with the presence of *Klebsiella* spp. in sample no 1 & 2 and the *Pseudomonas* spp in sample no 3.The identities of the pure cultures were further confirmed with few biochemical tests as shown in Table 2. *Staphylococcus* spp. were found to be present in 12 (80%) out of 15 eye and ear drop samples. Nine (60%) samples were found to harbor *Bacillus* spp. The prevalence of *Staphylococcus* spp. and *Pseudomonas* spp. in the samples is assumptive of bacterial shedding from floor and hands of handler during the preparation of drugs and hence indicates that the microbial quality of the products is not in line with the recommended standard[39-44].

Table 1: Prevalence of Pathogenic Microorganisms in Eye & Ear Drops

Sample No.	Sample Name	Total Aerobic Count (cfu/ml)	Total Fungal Count (cfu/ml)	Total Fecal Coliform Count (cfu/ml)	*Escherichia coli*	*Bacillus* Spp.	*Klebsiella* Spp.	*Salmonella* & *Shigella* Spp.	*Pseudomonas* Spp.	*Staphylococcus* Spp.
01`	**Cero**	4.7×10^2	3.0×10^1	–	–	–	+	–	–	–
02	**Livacin**	3.2×10^2	–	–	–	–	+	–	–	–
03	**Gento-HC**	8.5×10^2	1.1×10^2	–	–	–	–	–	+	+
04	**Dexon**	2.3×10^4	3.0×10^1	–	–	+	–	–	–	+
05	**Cloram**	3.2×10^2	–	–	–	+	–	–	–	+
06	**Sonexa-C**	6.7×10^2	1.2×10^2	–	–	+	–	–	–	+
07	**Lubric 0.5%**	3.4×10^2	5.0×10^1	–	–	+	–	–	–	+
08	**Hyprosol**	3.8×10^3	6.0×10^1	–	–	+	–	–	–	+
09	**Hypersol 5**	2.7×10^4	3.0×10^1	–	–	+	–	–	–	+
10	**AFM**	3.9×10^4	3.0×10^1	–	–	+	–	–	–	+
11	**Metadaxan**	1.8×10^4	–	–	–	+	–	–	–	+
12	**Methasol-N**	1.1×10^2	1.1×10^2	–	–	–	–	–	–	–
13	**Optear**	3.8×10^4	–	–	–	+	–	–	–	+
14	**Itchin-DS**	5.4×10^2	8.0×10^1	–	–	–	–	–	–	+
15	**Lubtear**	1.7×10^3	–	–	–	–	–	–	–	+

*USP Limit- $<10^2$ cfu/ml

\+ Presence of Bacteria

\- Absence of Bacteria

Table-2: Confirmative biochemical tests

Isolate	TSI				Motility	Indole	MR	VP	Citrate	Catalase	Oxidase	Suspected Organism
	Slant	Butt	H_2S	Gas								
01	**A**	**A**	**+**	**+**	**-**	**-**	**+**	**-**	**+**	**+**	**+**	***Klebsiella* spp.**
02	**K**	**A**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	***Pseudomonas* spp.**
03	**K**	**A**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	***Staphylococcus aureus***
04	**A**	**A**	**-**	**+**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	***Bacillus cereus***

A= Acidic Reaction, K= Alkaline Reaction, MR= Methy Red, VP= Voges-Proskauer, += Positive, -= Negative

Antimicrobial activity

Thirteen (13) samples (88.66%) exhibited the anti-bacterial activity against the relevant model organisms. Of the 15 samples studied, sample 1 exhibited pronounced antibacterial activity (23.8-42.5 mm) against *E. coli*, *Salmonella* spp, *Staphylococcus* spp, *Listeria* spp, *Vibrio* spp, *Bacillus* spp, *Pseudomonas aereginosa*; and moderate activity (13 mm) against *Klebsiella* spp (Table 3). Sample 5 showed pronounced antibacterial activity (24.2 mm) against *E. coli*, *Salmonella* spp, *Vibrio* spp; whereas higher activity (19.1-21.5 mm) against *Listeria* spp and *Pseudomonas aereginosa*; and moderate activity (14.6 mm) against *Staphylococcus* spp. No activity against *Klebsiella* spp. was scored. Other samples and the reference antibacterial drugs were also showed significant inhibitory activities against the model organisms (Table-3).

Table – 3: Anti-bacterial activity of the samples studied.

Bacterial	Samples														
	Cero	Livacin	Gento-HC	Dexon	Cloram	Sonexa-C	Lubric 0.5%	Hyprosol	Hypersol-5	AFM	Metadaxan	Methasol-N	Optear	Itchin-DS	Lubtear
1	31.2	32.5	22.5	0	24.2	25.5	5.1	9.1	0	11	0	25.5	0	13.1	11.4
2	25.6	26	20.39	0	7.0	7.8	10.6	9.0	0	0	0	27.0	7	10.7	9.8
3	27.6	28.8	18.26	0	14.6	8.5	13	12.1	0	8.6	0	24.3	0	0	8.5
4	32.5	29.9	25.1	0	21.5	24.1	8.7	9.1	0	0	0	0	23.5	0	10.4
5	13	24.2	0	0	0	0	13.8	0	0	0	0	19.0	0	11.2	0
6	32.3	31.8	23	0	24.2	23.3	7.7	8.3	0	0	0	22.5	0	10.3	0
7	42.5	38	21.7	14.7	24.2	26	0	0	0	0	0	29.4	14.2	0	0
8	23.8	25.25	14.54	0	19.1	18.9	0	0	0	0	0	18.3	0	0	0

Strain 1: *Escherichia coli*, Strain 2: *Bacillus* spp., Strain 3: *Staphylococcus* spp., Strain 4: *Pseudomonas aereginosa*
Strain 5: *Klebsiella*spp., Strain 6: *Salmonella* spp., Strain 7: *Vibrio* spp., Strain 8: *Listeria* spp.
Positive Control: Gentamicin (Samples: 1-6) & Streptomycin (Samples: 7-15)

CONCLUSION

Microbial control of pharmaceuticals is concerned with minimizing the risk of drug usage. As has been conducted in the current investigation, further simulation of such microbial contamination in drug samples above the safety limit may pose serious obstacle for diseases medication. Good hygiene practices, proper handling, clean environment are necessary for avoiding microbial contamination and maintenance of drug quality. To ensure the patient safety as well as to maintain the public health harmony, a customary microbiological examination of

sterile drugs is suggested, especially in the developing countries, where the ease of microbial contamination is usual. The antimicrobial activity tested in the current research poses another aspect of demonstration of the effectiveness of drugs in mitigating diseases.

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Conflict of Interest

Authors have no potential conflict of interests.

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